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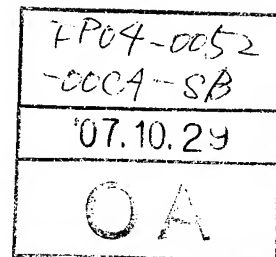
(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Variety Classification Method for Barley or Malt Using
Gene Diagnosis and the Primer Used Therefor

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(57) 4 Claims

Notice: This application is as filed and may therefore contain an
incomplete specification.

Industrie Canada   Industry Canada

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What is claimed is:

1. A method for classifying a variety of barley or malt by amplifying genomic DNA of barley or malt by PCR with a primer comprised of a sequence complementary to a gene that is important for brewing, and examining a difference in base sequence of said DNA.

2. A method for classifying a variety of barley or malt by amplifying genomic DNA of barley or malt by polymerase chain reaction (PCR) with either a set of oligonucleotides consisting of the sequence of (1) 5'-TTCAAAGCAGCAGCAGCG-3' (Seq ID No. 1) and (2) 5'-TTCTTCTGGTGCGCTCATC-3' (Seq ID No. 2) or a set of oligonucleotides composed of the sequence complementary to said sequence as an essential primer, and also with either a set of oligonucleotides consisting of the sequence of (3) 5'-ATAAGTGGGCATCAATTCGGC-3' (Seq ID No. 3) and (4) 5'-GTGTGTCTGGCCAGGTAT-3' (Seq ID No.4) or a set of oligonucleotides composed of the sequence complementary to said sequence as a selective essential primer, and using either set of said essential primer with either set of said selective essential primer or either strand thereof, and by examining a difference in the base sequence of said amplified DNA.

3. In the method described in Claim 1, a method for classifying the variety of barley or malt by amplifying the genomic DNA by PCR with oligonucleotides consisting of the

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Title of the Invention

Variety classification method for barley or malt using gene diagnosis and the primer used therefor

Field of the invention

The present invention relates to a method for classifying the variety of malting barley or malt using gene diagnosis and primers used for said method.

Description of the Related Art

For variety classification of barley and malt, there has been conventionally used a method for classifying the variety by comparing an SDS polyacrylamide gel electrophoretic pattern of hordein and esterase contained therein. In addition, a classification method using gene diagnosis has recently been developed (e.g., Chee et al., J. Am. Soc. Brew. Chem., 51, 93 (1993)).

However, the variety classification method by way of comparing the electrophoretic pattern of hordein and esterase is not necessarily to be an accurate classification method, because the electrophoretic pattern may be modified according to growing conditions of barley or due to the degradation of the hordein and esterase by protease during malting process. Furthermore, since most of classification methods using gene diagnosis use genes from unidentified origin as probe or primer, there has been a problem that results obtained by the method cannot be directly correlated

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Table 1

Targeted gene	Primer sequence
β-Amylase	(1) 5'-TTCAAAGCAGCAGCAGCG-3'
	(2) 5'-TTCTTCTGGTGCGCTCATC-3'
α-Amylase	(3) 5'-ATAAGTGGGCATCAATTCGGC-3'
	(4) 5'-GTGTGTCTGGCCAGGTAT-3'
β-Glucanase	(5) 5'-CGTGAAAAAACCGCCGCCGA-3'
	(6) 5'-CTTTCTCTCTCTAGCTGCGT-3'
B1-Hordein	(7) 5'-CCACCATGAAGACCTTCCTC-3'
	(8) 5'-TCGCAGGATCCTGTACAACG-3'

The present invention also provides primers used for the variety classification method. Primers according to the present invention can be synthesized with a commercial automated DNA synthesizer using the β-cyanoethylphosphoamidide method or thiophosphite method.

More precisely, the present invention provides a variety classification method for barley or malt comprising amplification of the genomic DNA of barley or malt by PCR with the primer having the base sequence complementary to the gene which is important gene in the brewing, and examination of the difference of base sequence of the amplified DNA (Claim 1).

The present invention also provides a variety classification method for barley or malt comprising the

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essential primers and the selective essential primers, a combination of at least any one or any two primers from the group of selective primers, of and the classification of a variety of barley or malt based on the difference in the base sequence of the amplified DNA (Claim 3).

Furthermore, the present invention provides PCR primers comprising oligonucleotides consisting of the sequences 1-6 shown in the sequence listing table, or those consisting of sequences complementary to them (Claim 4).

According to the present invention, the genomic DNA is first extracted from the sample of barley or malt. Extraction of the genomic DNA may be carried out, for example, by a CTAB method (Nucleic Acids Res., 8, 4321 (1980)). Then, a portion of the targeted gene is amplified by applying the primer of the present invention to the genomic DNA. The partial amplification of the genomic DNA may be carried out, for example, by PCR (Science, 230, 1350 (1985)). Then, the variety of barley or malt is classified either by the base sequence determination of amplified DNA thus obtained or based on the difference in the base sequence detected by electrophoresis on denatured gradient gel or temperature gradient gel, or on the restriction enzyme cleavage pattern.

Since the method of the present invention aims to target the gene which is important for brewing, it is highly

Fig. 4 is a photography of polyacrylamide gel electrophoretic pattern of DNAs which are amplified by PCR with primers (7) and (8) (left half, A ～ E) and those which were then treated with restriction enzyme HaeIII (right half, A ～ E). In the figure, A, B, C, D and E denote the type of polyacrylamide gel electrophoretic pattern, and M is DNA MW marker 9 (Nippon Gene) and M' DNA MW marker 2 (Nippon Gene).

Detailed Description of the Invention

The invention will now be described with reference to specific examples, however, it should understood that the technical scope of the invention is not to be construed as being limited to them in any way.

Example 1

Extraction of the Genomic DNA

In this embodiment, as the variety of barley or malt, Amagi Nijo (called Variety No. 1 hereinafter), Haruna Nijo (called Variety No. 2 hereinafter), Misato Golden (Variety No. 3 hereinafter), Clipper (called Variety No. 4 hereinafter), Schooner (called Variety No. 5 hereinafter), Stirling (called Variety No. 6 hereinafter), Harrington (called Variety No. 7 hereinafter), Manley (called Variety No. 8 hereinafter), Ellice (called Variety No. 9 hereinafter) and Alexis (called Variety No. 10 hereinafter)

A PCR mixture (100 μl) which contained the genomic DNA
(100 ng) extracted from 10 barley grains of each variety,
dNTPs (20 nmol each), primers (1) and (2) (10 pmol each) and
Taq DNA polymerase (2.5 U) was subjected to 33 cycles of
reaction wherein each cycle consisted of incubating the
mixture in sequence at 94°C for 1 min, 55°C for 2 min, and
72°C for 1 min, and then finally treated at 72°C for 5 min.
After the completion of the PCR, restriction enzymes NcoI
and EcoT22I (5 U each) and a buffer for the enzymatic
reaction were added to the reaction mixture (8 μl), and the
mixture was incubated at 37°C for 1 h. This reaction
mixture was electrophoresed on 5% polyacrylamide gel. After
the electrophoresis, the gel was stained with ethidium
bromide, and then the DNA were made visible by UV exposure.
Results are shown in Fig. 1. As shown in this figure, from
the electrophoretic pattern of the fragments of DNAs
obtained by digestion with restriction enzymes NcoI and
EcoT22I, 10 varieties of barley could be classified into 5
types (A, B, B', C and C'). Furthermore, based on results
of analyses on single grains, Variety Nos 5 and 10 were
found to be a mixed type consisting of either C and C' or B
and C, therefore denoted as C/C' and B/C respectively.

Example 4

Variety classification method using primers (3) and (4)

Analysis was performed under similar conditions to those

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digested with the restriction enzyme HaeIII under the similar conditions to those described for Example 3, and electrophoresed. The fragment patterns were as shown on the right half of Fig. 4. By comparing the results from intact and digested PCR products, as shown in Fig. 4, the electrophoresis pattern could be classified into 5 types (A, B, C, D and E).

Example 7

Variety classification method by overall evaluation

Results of the type classification performed in Examples 3 ~ 6 are summarized in Table 2. These results show that it is possible to classify all of the 10 variesties by using the overall evaluation.

Table 2 Type classification

Variety No. PCR Primers	1	2	3	4	5	6	7	8	9	10
(1), (2)	A	A	B	C'	C/C'	C	B	B'	B	B/C
(3), (4)	A	B	C	C	A	A	C	C	A	A
(5), (6)	A	A	B	A	B	B	A	A	B	B
(7), (8)	A	A	A/B	C	B	D	B	A	B	A/C/E

classification can be carried out from the viewpoint of

breeding of barley for brewing and the quality control of

materials used for brewing.

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : DNA

SEQUENCE DESCRIPTION : GTGTGTCTGG CCAGGTAT   18

SEQ ID NO : 5

SEQUENCE LENGTH : 20

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : DNA

SEQUENCE DESCRIPTION : CGTGAAAAAA CCGCCGCCGA   20

SEQ ID NO : 6

SEQUENCE LENGTH : 20

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : DNA

SEQUENCE DESCRIPTION : CTTTCTCTCT CTAGCTGCGT   20

SEQ ID NO : 7

SEQUENCE LENGTH : 20

SEQUENCE TYPE : nucleic acid
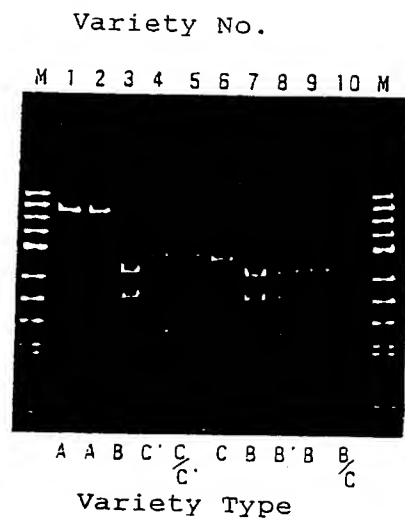
STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : DNA

SEQUENCE DESCRIPTION : CCACCATGAA GACCTTCCTC   20

SEQ ID NO : 8

Fig. 1

Variety No.

M 1 2 3 4 5 6 7 8 9 10 M

A A B C C C C B B B B B/C
       /C   /C

Variety Type

Fig. 3

Variety Type

M A B